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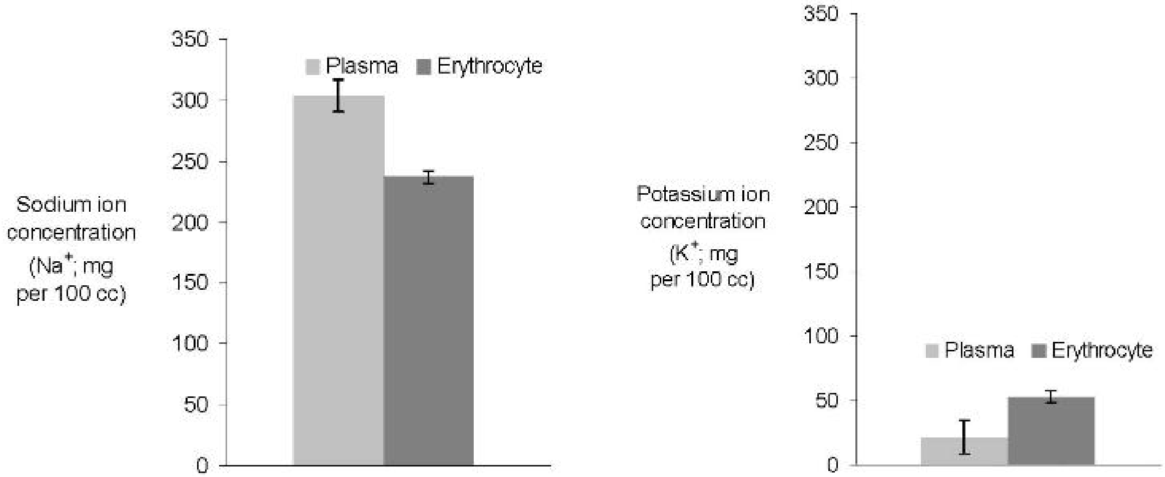
Early Evidence for the Semi-Permeable Cell Membrane

Imagine it's the late 1800's and an exciting time in cell biology as little is known about the biochemical and physical composition, structure and organization of cells and particularly cell membranes. The current state of knowledge at this time, based in part on work conducted on erythrocytes (red blood cells) as early as 1773, can be summarized as follows (De Weer, 2000):

- Living cells contain fluid.
- This fluid is separated from the external environment by a visible membrane of unknown composition and structure.
- The volume of this fluid can change depending upon the nature of the fluid in which cells are immersed. A change in volume can cause the cell to either shrink, swell or sometimes burst (lyse).
- The membrane is permeable to water but apparently not generally to solutes.

An example of the kind of data that supported the contention that cell membranes are impermeable to solutes appears in Figure 1 (Jacobs, 1931). Consider the data and answer the questions that follow.



Average concentration of free (unbound) 1) sodium (Na+) and 2) potassium (K+) ions in live dog erythrocytes (dark gray, mg per 100 cc) and the blood plasma in which the erythrocytes are immersed (light

gray, mg per 100 cc). N=7. Error bars are 95% confidence intervals.
Data from Kerr (1929).

1. Review Figure 1. Please describe what the data suggest about the concentration of a) sodium and b) potassium ions inside dog erythrocytes relative to the surrounding plasma.

2. Data from other mammal species reveal a pattern similar to that above (Jacobs, 1931). Please explain why data like these suggest that cell membranes are impermeable to (at least some) solutes.

3. What would you expect the figures above to look like if the membrane of erythrocytes was permeable to these two solutes? That is, what is the null hypothesis for a comparison of the relative concentration of a) sodium and b) potassium ions in an erythrocyte to that of the surrounding plasma? Sketch each figure above and add your null hypotheses to each sketch.

Works Cited

- De Weer, P. 2000. A century of thinking about cell membranes. *Annual Review of Physiology.* 62:919-926.
- Jacbos, M.H. 1931. Diffusion processes in non-living and living systems. *Proceedings of the American Philosophical Society.* 70:167-186.
- Kerr, S.E. 1929. Studies on the inorganic composition of blood. III. The influence of serum on the permeability of erythrocytes to potassium and sodium. *Journal of Biological Chemistry.* 85: 47-64.

What Questions Does the Selective Movement of Materials Across the Cell Membrane Inspire

Like you, scientists of the 19th century understood the process of diffusion, however, perhaps unlike you, they did not know a lot about the cell membrane. In fact, the current state of knowledge at this time, based in part on work conducted on erythrocytes (red blood cells) as early as 1773, can be summarized as follows (De Weer, 2000):

- Living cells contain fluid.
- This fluid is separated from the external environment by a visible membrane of unknown composition and structure.
- The volume of this fluid can change depending upon the nature of the fluid in which cells are immersed. A change in volume can cause the cell to either shrink, swell or sometimes burst (lyse).
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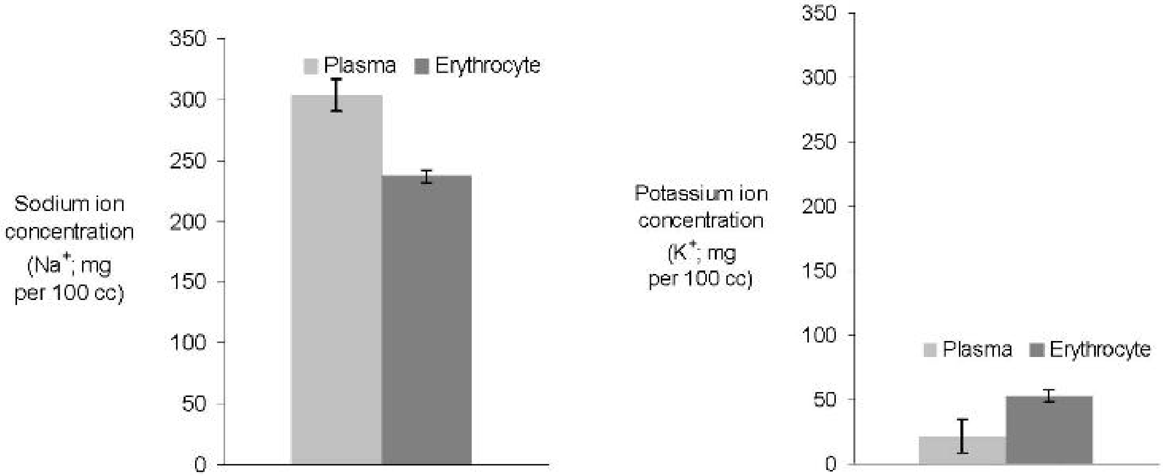


Average concentration of free (unbound) 1) sodium (Na+) and 2) potassium (K+) ions in live dog erythrocytes (dark gray, mg per 100 cc) and the blood plasma in which the erythrocytes are immersed (light gray, mg per 100 cc). N=7. Error bars are 95% confidence intervals. Data from Kerr (1929).

As you consider what was known about cell membranes in the late 1800’s (described above), imagine you are a 19th century cell biologist. What kinds of questions does this information (including Figure 1) inspire you to ask about the structure (molecular composition and organization) and function (job) of the cell membrane? Record at least two questions on a separate piece of paper.

Summary of Simple Diffusion

- Diffusion is the **net** movement of molecules from areas of higher concentration to areas of lower concentration. That is, over time molecules redistribute such that the average direction of molecular movement (i.e. 'net' movement) is away from areas of high concentration toward areas of lower concentration, i.e. along or 'down' a gradient of (decreasing) concentration.
- Importantly, diffusion refers to the redistribution of molecules/ions **of a single type**. For example, we can talk about the diffusion of water molecules (i.e. osmosis) or we can talk about the diffusion of sodium ions dissolved in water. In reality, both types of molecules will be diffusing, assuming these two substances are not equally distributed throughout the solution. However, when we discuss diffusion we describe the net directional movement of a single type of molecule or ion, the sodium ion for example.
- Diffusion occurs because all molecules (particles) are in constant random motion due to their inherent energy (heat). As a result, molecules randomly move into and out of a given space (volume) within a fluid. (Imagine for example molecules moving into and out of a 1cm x 1cm x 1cm volume.) Because molecular motion is random, the numbers of molecules moving into relative to out of this volume will depend upon the 'supply' or local concentration of molecules. If molecules are more abundant in the volume than in surrounding volumes, then more molecules are available to wander out of as opposed to into the volume in a given period of time. Thus, even though molecules will move both into and out of the volume during that period of time, the overall or net direction of molecule movement

will be out of that volume, i.e. from an area of higher concentration toward an area of lower concentration.
- Over time, unequal rates of 'entry versus exit' cause the number of molecules to decline in areas/volumes of high molecule density and to increase in areas of relatively low molecular density.
- Eventually, these processes redistribute molecules within the fluid such that the number of molecules moving into and out of any given volume is equal throughout the fluid. (Unless other factors are operating to prevent molecules from moving in this way.) This is equilibrium.
- Thus, diffusion - the **net** movement of molecules - ceases when the concentration of a molecular species is equal throughout the solution. Importantly, individual molecules continue to move but the overall concentration of molecules remains constant throughout the solution over time.
- Finally, diffusion is a property of a 'population' of molecules not individual molecules. Individual molecules do not diffuse because their movements are random; individual molecules do not trace a path from an area of higher concentration to an area of lower concentration. Rather, diffusion is the overall redistribution of a huge number of unequally distributed molecules from areas where they are more concentrated toward areas where they are less concentrated which results from the collective random movement of these particles.

Works Cited

- De Weer, P. 2000. A century of thinking about cell membranes. *Annual Review of Physiology.* 62:919-926.
- Jacbos, M.H. 1931. Diffusion processes in non-living and living systems. *Proceedings of the American Philosophical Society.* 70:167-186.
- Kerr, S.E. 1929. Studies on the inorganic composition of blood. III. The influence of serum on the permeability of erythrocytes to potassium and sodium. *Journal of Biological Chemistry.* 85: 47-64.

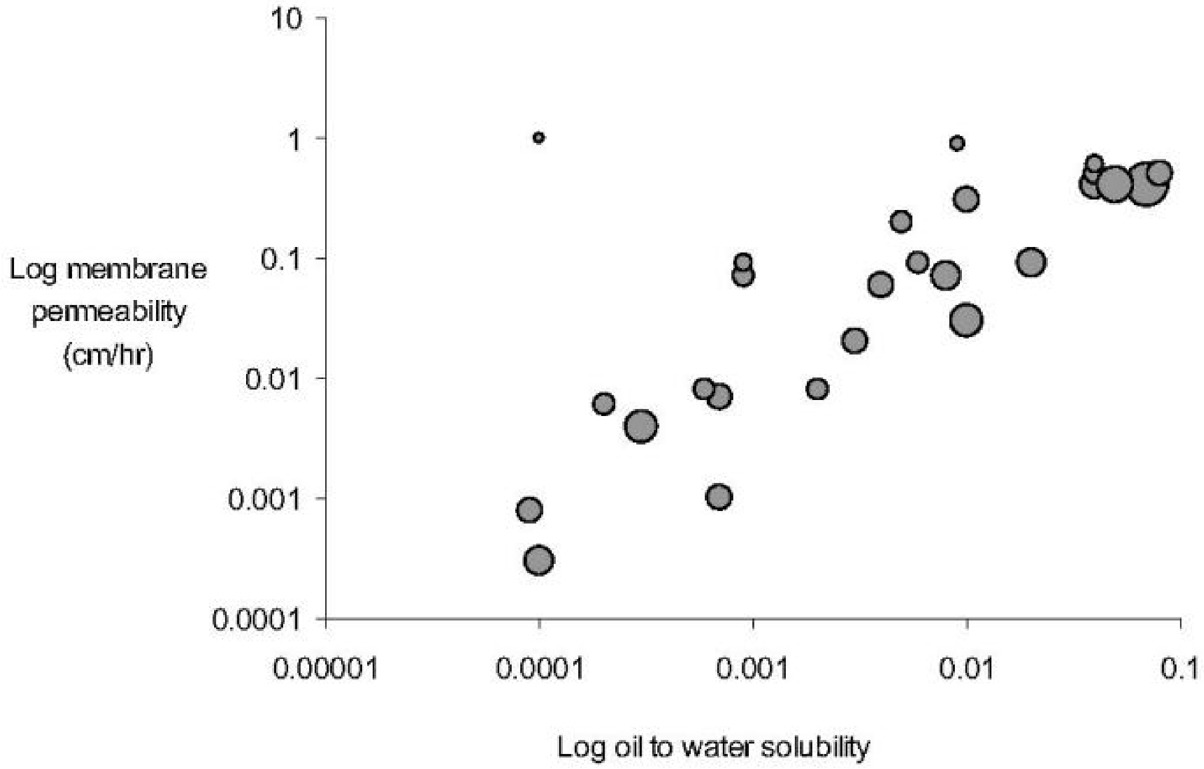
Deducing the Biochemical Composition of the Cell Membrane - Reasoning from Quantitative Evidence

Just like you, early investigators had lots of questions about the structure and chemical composition of the cell membrane and how these properties could account for differences in the membrane's permeability to different type of molecules.

At least one of these researchers, Ernest Overton of the University of Zurich, realized that systematic differences in the permeability of the membrane to solutes that varied in size, charge and lipid solubility (how well they dissolved in lipids relative to water) could provide insight into the membrane's biochemical composition. That is, information about the membrane's permeability to molecules of varied chemical properties could reveal biochemical properties of the biological molecules forming the membrane itself.

After investigating the permeability of a large variety of cell types to hundreds of different compounds, Overton eventually deduced the general chemical properties of the cell membrane and from this correctly predicted the type of biological molecule from which the membrane must be primarily formed (De Weer, 2000)! Interestingly, and as so often happens in science, Overton's original research question had nothing to do with investigating properties of the cell membrane. Rather his pursuit of this question bloomed from research on heredity for which he needed a traceable material that would pass through the plant cell membrane (Eichman, 2007).

Unfortunately, Overton never published the detailed results of these studies so his data are no longer with us (De Weer, 2000). Data similar to Overton's do exist, however, and an example taken from a paper published in 1937 based on work conducted on alga cells is presented in figure below.



Relationship between the permeability (log cm/hr) of the plasma membrane of cells of the alga *Chara ceratophylla* to non-ionic, organic solutes and the solute's a) size as indicated by the relative size of the bubble (molecular mass; grams/mole); the larger the bubble the larger the solute and b) solubility in oil relative to water (log). Low values of oil to water solubility (ex. 0.001) indicate virtually no solubility in lipids and very high solubility in water. Increasing values indicate an increasing solubility in oil relative to water. Data from Collander (1937).

What do these data suggest to you about the biochemical properties of the cell membrane and, in turn, the type of biological molecule from which the cell membrane is constructed? To answer this question, review the figure above and work through the questions below.

1. a. Carefully review the figure legend, axes labels and the data presented. In a sentence or two, describe what relationship, if any, there is between

membrane permeability and the oil to water solubility of a solute.

2. Describe what relationship, if any, there is between cell permeability and solute size. To answer this question, work through the series of questions below.

- a) On a piece of paper, sketch what the above figure is expected to look like if permeability increases with increasing solute size. (Assume the relationship between permeability and solubility is the same as above.)
- b) On a piece of paper, sketch what the above figure is expected to look like if permeability decreases with increasing solute size. (Assume the relationship between permeability and solubility is the same as above.)
- c) Does the actual relationship between permeability and solute size match either hypothesis you sketched in response to parts a and b? Please explain.
- d) What do the data presented in figure 1 and your answer to part c suggest about the relationship between cell permeability and solute size? Please explain in a sentence or two.

3. Consider what you know about hydrophobic and hydrophilic molecular interactions together with the results you described in questions 1 and 2 above. What do these results collectively suggest, if anything, about the type of biological molecules that form the cell membrane (i.e. the biochemical composition)? Please explain how the data support your conclusion.

4. An undisturbed cell membrane is generally impermeable to ions. Does your description of the cell membrane from question 3 account for this? Please explain.

5. If your description of the cell membrane cannot account for the observation that undisturbed cell membranes are generally impermeable to ions, please amend it so that it does.

6. Early on scientists knew that water, a **very small** polar molecule, could diffuse across the membrane although this problem was ignored for many

years. Can your description of the properties of the cell membrane the account for the cell membrane’s permeability to water? Yes or no? Please explain.

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- Collander, R. 1937. The permeability of plant protoplasts to non-electrolytes. *Transactions of the Faraday Society.* 33:985-990.
- De Weer, P. 2000. A century of thinking about cell membranes. *Annual Review of Physiology.* 62:919-926.
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An Emerging Model of Cell Membrane Structure

In a series of papers interpreting results not unlike these published in the 1890's, Ernest Overton correctly predicted that cell membranes were composed primarily of lipid molecules and therefore were semi-permeable (De Weer, 2000). Lipid soluble, non-polar molecules could diffuse through the membrane while water soluble, polar molecules and ions were generally unable. Although Overton's hypothesis was initially controversial because it opposed current scientific opinion (Eichman, 2007), his conclusions were correct and his interpretation of membrane structure would be enriched and modified over time.

One important discovery that further clarified cell membrane structure was by made by Dutch scientists E. Gorter and F. Grendel, who in the early 1920’s, compared the surface area of plasma membranes extracted from erythrocytes (red blood cells which they refer to as chromocytes) to the surface area of the cells themselves. To do this, they isolated erythrocytes from the plasma of several animals species and used pure acetone (an organic solvent) to separate the lipid membrane from the aqueous cytoplasm. Follow up extraction on the cytoplasm component produced 'only small traces of lipoid [lipid] substances' (Gorter and Grendel, 1925, p.439) indicating that the acetone extraction had successfully retrieved all membrane. Their results, as presented in their 1925 paper, appear in Figure 1.

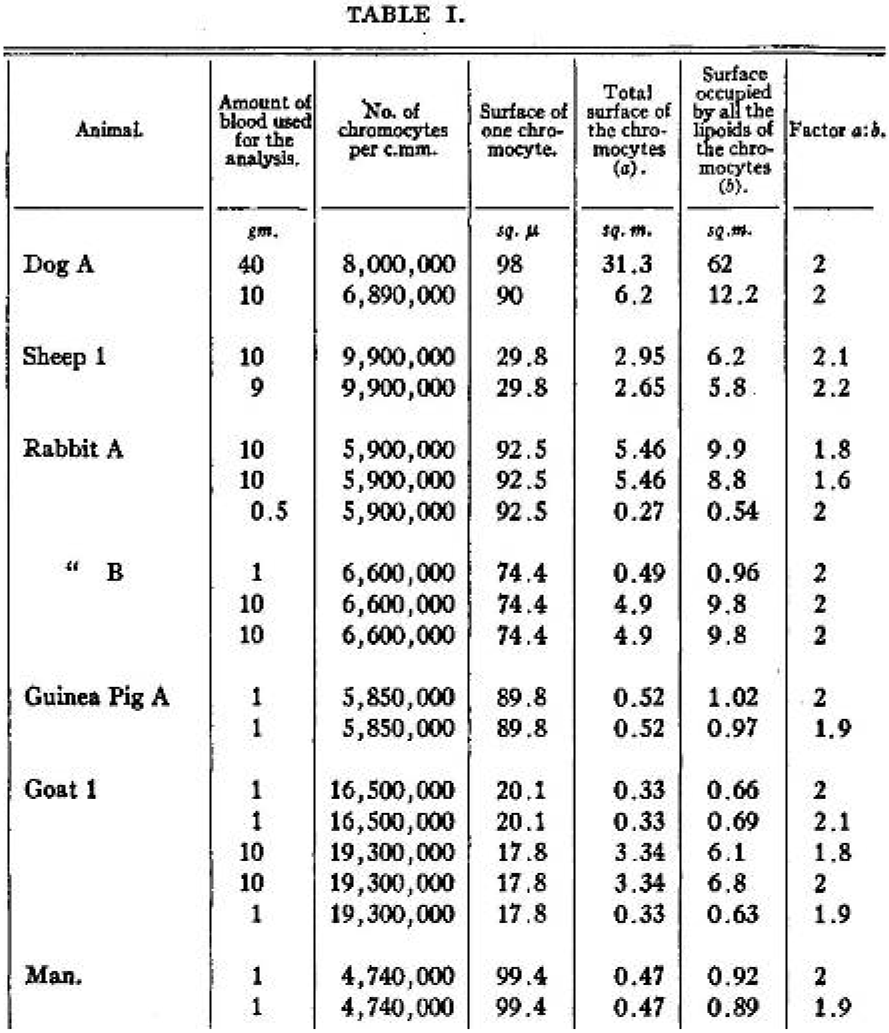


TABLE I.

Animal.	Amount of blood used for the analysis.	No. of chromocytes per c.mm.	Surface of one chromocyte.	Total surface of the chromocytes (*a*).	Surface occupied by all the lipoids of the chromocytes (*b*).	Factor *a*:*b*.
	gm.		sq. µ	sq. m.	sq. m.	
Dog A	40	8,000,000	98	31.3	62	2
	10	6,890,000	90	6.2	12.2	2
Sheep 1	10	9,900,000	29.8	2.95	6.2	2.1
	9	9,900,000	29.8	2.65	5.8	2.2
Rabbit A	10	5,900,000	92.5	5.46	9.9	1.8
	10	5,900,000	92.5	5.46	8.8	1.6
	0.5	5,900,000	92.5	0.27	0.54	2
" B	1	6,600,000	74.4	0.49	0.96	2
	10	6,600,000	74.4	4.9	9.8	2
	10	6,600,000	74.4	4.9	9.8	2
Guinea Pig A	1	5,850,000	89.8	0.52	1.02	2
	1	5,850,000	89.8	0.52	0.97	1.9
Goat 1	1	16,500,000	20.1	0.33	0.66	2
	1	16,500,000	20.1	0.33	0.69	2.1
	10	19,300,000	17.8	3.34	6.1	1.8
	10	19,300,000	17.8	3.34	6.8	2
	1	19,300,000	17.8	0.33	0.63	1.9
Man.	1	4,740,000	99.4	0.47	0.92	2
	1	4,740,000	99.4	0.47	0.89	1.9

Original data table from Gorter and Grendel (1925) reporting the results of experiments in which the plasma membrane was extracted from erythrocytes ('chromocytes') of a variety of species and its surface area compared to that of the cells themselves. The ratio of the plasma membrane surface area to cell surface area appears in the final column.

What does Gorter and Grendel's data suggest about the physical organization of the plasma membrane? To answer this question, review Figure 1 and the answer the questions below.

1. Carefully examine the table in Figure 1. In a sentence or two describe what Gorter and Grendel's results suggest about the surface area of the erythrocyte plasma membrane relative to that of the cell itself.

2. Develop a model of the physical organization of the cell membrane that explains this result. Please make sure to explain how the data support your model.

3. On the basis of the data presented above, Gorter and Grendel concluded that *'the chromocytes [erythrocytes] of different animals are covered by a layer of lipoids [lipids] just two molecules thick.'*

Compare your interpretation of their data (i.e. your model described in question 2) to their interpretation of their data. How are they similar? How do they differ? If they differ substantially, explain both how you would revise your model and why these revisions make biological/biochemical sense.

4. The success of some experiments depends a great deal on the system (ex. organism) one was chooses to study because not all systems will produce equally clear results. For example, Mendel's choice to study inheritance in pea plants facilitated his discovery of simple inheritance because, in contrast to many organisms, pea plants have a large number of simply inherited traits. Specifically, the system's simplicity promoted mathematical quantification, which was key to revealing the pattern of inheritance and from this inferring possible responsible mechanisms, while the diversity of traits enabled him to see these patterns repeatedly. Pea plants also grow easily and quickly which enabled him to gather huge amounts of data relatively quickly. It is, for example, impossible to imagine Mendel making the same discoveries using elephants as his model organism!

One might argue that Gorter and Grendel made a similarly wise choice because, unlike nearly all eukaryotic cells, mature mammalian erythrocytes lack organelles; they have no nucleus, mitochondria, Golgi bodies, etc. Please explain why the lack organelles was important to their success. Be sure to describe the effect this had on the mathematical relationships they found.

Works Cited

- De Weer, P. 2000. A century of thinking about cell membranes. *Annual Review of Physiology.* 62:919-926.
- Eichman, P. 2007. http://www1.umn.edu/ships/9-2/membrane.htm. SHiPS Resource Center for *Sociology, History and Philosophy in Science Teaching*
- Gorter, E. and F. Grendel. 1925. On bimolecular layers of lipoids on the chromocytes of the blood. 439-443. *The Journal of Experimental Medicine.* 41:439-443.

Definitions

- **model** - in science, a testable explanation for a process or pattern; a testable description of how a process works or explanation describing a cause of a pattern.

A Lipid Bilayer Model of Cell Membrane Structure

On the basis of data, as well as independent evidence that lipids could form two layered structures (Gorter and Grendel, 1925), Gorter and Grendel correctly concluded that the cell membrane was not one molecule thick as proposed by Irving Langmuir in 1917 but rather two. That is, the phospholipid molecules that formed the cell membrane were arranged in two layers to form a lipid ‘bilayer’.

In this same paper, Gorter and Grendel (1925) also correctly predicted the physical orientation of the phospholipid molecules within the bilayer. Like Ernest Overton before them, their prediction was based on their understanding of molecular interactions.

To generate a model of how phospholipid molecules are organized into a bilayer, based on your own understanding of molecular interactions, answer the questions below.

1. Examine the phospholipid molecule depicted here. In light of its structure and the fact that both the interior and exterior surfaces of a cell’s plasma membrane physically contact aqueous (water based) solutions, cytoplasm and interstitial fluid respectively, suggest a model to explain how phospholipid molecules are arranged to form a bilayer. Sketch your model and, in a couple sentences, explain the reasoning on which your model is based.

2. How does your model compare to that proposed by Gorter and Grendel (1925), which is now a part of our current model of membrane structure? To answer this question, compare your proposal to Gorder and Grendel's original description of the organization of individual lipid molecules within the bilayer and answer the associated questions.

`...the chromocytes [erythrocytes] of different animals are covered by a layer of lipoids [lipids] just two molecules thick...every chromocyte is surrounded by a layer of lipoids, of which the polar groups are directed to the inside and to the outside [of the cell] in much the same way as`

`Bragg (1) supposes the molecules to be oriented in a 'crystal' of a fatty acid, and as the molecules of a soap bubble are according to Perrin (2). On the boundary of the two phases, one being the watery solution of hemoglobin, and the other the plasma, such an orientation seems a priori to be the most probable one.(Gorter and Grendel, 1925, p.439)`

Draw a sketch illustrating Gorten and Grendel's description of how lipids are organized within the bilayer and then compare your model to theirs. How are they similar? How do they differ? If the two models differ substantially, explain both how you would revise your model and why these revisions make biological/biochemical sense.

3. Gorter and Grendel are fairly confident they have correctly described the organization of the lipid membrane. How do they support their conclusion about the orientation of the lipids? Please explain.

Works Cited

- Gorter, E. and F. Grendel. 1925. On bimolecular layers of lipoids on the chromocytes of the blood. 439-443. *The Journal of Experimental Medicine*. 41:439-443.
- Langmuir, I. 1917. The constitution and structural properties of solids and liquids. II. Liquids. *Journal of the American Chemical Society. 39: 1848-1906.*

The Fluid Mosaic Model of the Cell Membrane - The Mosaic

Although the bilayer nature of the cell membrane was described in the mid-1920's, it was not until 1972 that the currently accepted model of the plasma membrane, the **fluid mosaic model**, was formally outlined by S. J. Singer and Garth L. Nicolson in the journal *Science*.

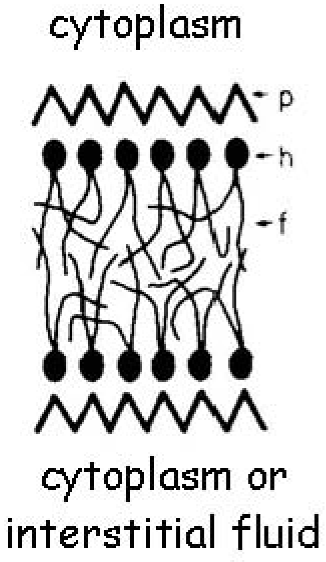
Singer's work on membrane structure originated in the 1950's when he, along with other protein chemists, demonstrated that many water-soluble proteins like those found in cytoplasm could unexpectedly dissolve in nonaqueous, non-polar solvents. Furthermore, the shape a protein assumed differed in hydrophobic and hydrophilic environments (Singer, 1992).

From an historical perspective these results are significant because they led Singer to wonder about the structure of the proteins revealed to be closely associated with lipid-rich, and therefore nonaqueous, cell membranes in the 1930's (Eichman, 2007). As he later wrote,

> Although we had not experimented with membrane proteins and knew very little about membranes at the time, as almost an aside we speculated [in a 1962 publication] that because "the cellular environment of many proteins contains high concentrations of lipid components in a wide variety of cellular membranes, the gross conformations of these proteins in situ may be determined by this association with a nonaqueous environment." This notion set off a train of ideas and experiments that eventually led us to the fluid mosaic model. (Singer, 1992, p.3)

At the time Singer's train set off, the standard model for membrane structure, the Davson-Danielli-Roberston (DDR) model, was a bilayer of lipids sequestered between two monolayers of unfolded protein (Figure 1).

Each protein layer faced an aqueous environment, cytoplasm or interstitial fluid, depending upon whether the membrane enclosed an organelle or the cell itself (Figure 1; Singer, 1992).



Original figure from Singer (1992) illustrating the Davson-Danielli-Robertson model of the plasma membrane. Notice that the lipid bilayer is isolated from the surrounding aqueous environment by two layers of unfolded membrane protein (p). Each membrane forming lipid is composed of a polar head group (h) and fatty acyl tail (f). Text added.

When Singer and colleagues applied their understanding of the influence of solvent environment on protein conformation specifically to the problem of membrane proteins, they realized the DDR model was energetically untenable. As he and Nicolson (1972) later wrote,

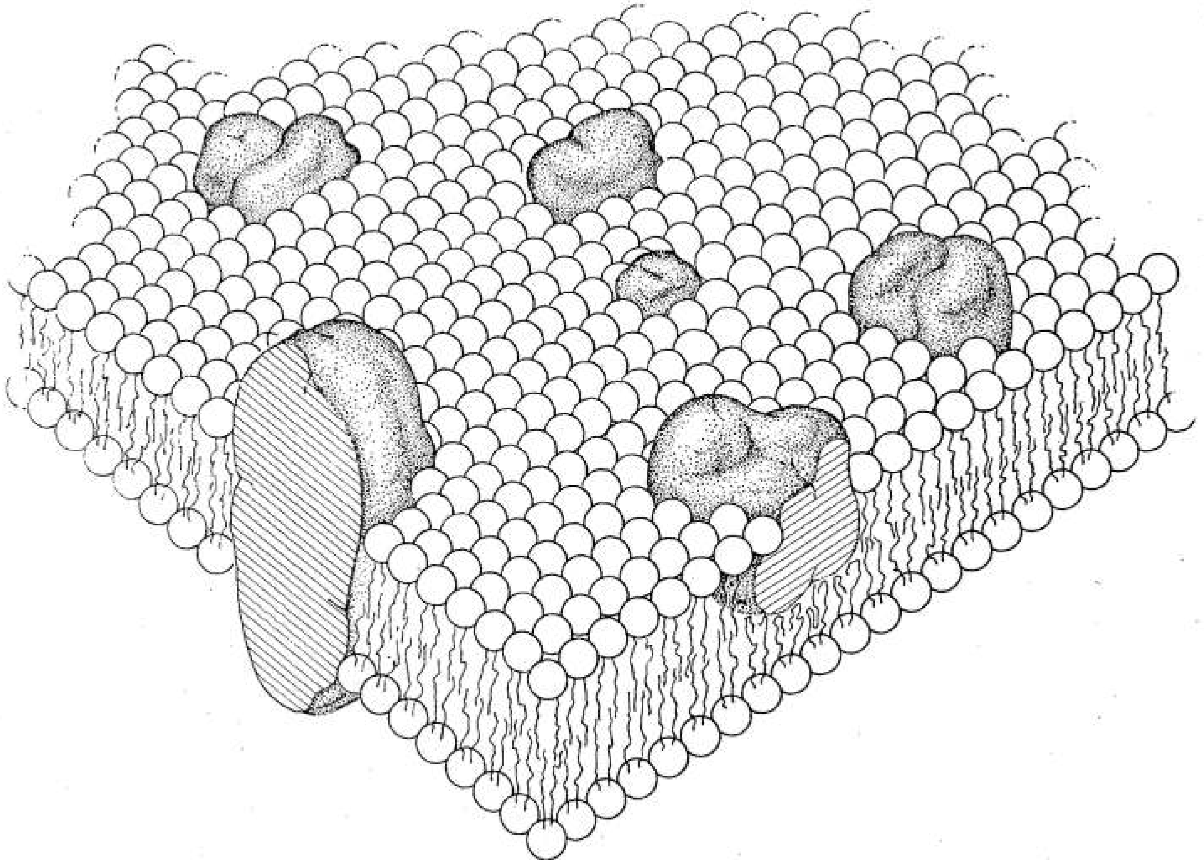
> The latter [DDR] model is thermodynamically unstable because not only are the non-polar amino acid residues of the membrane proteins in this model perforce [by circumstance] largely exposed to water but the ionic and polar groups of the lipid are sequestered by a layer of protein from contact with water. Therefore, neither hydrophobic nor hydrophilic interactions are maximized in the classical [DDR] model. (Singer and Nicolson, 1972, p.721)

That is, the DDR model was energetically unfeasible because the constitutive molecules could not stably persist in aqueous cytoplasm in the physical conformation proposed. Just as oil and water will spontaneously separate when left to stand after shaking, the hydrophilic and hydrophobic components of a single polypeptide or an entire cell will spontaneously organize so that hydrophilic elements are in contact with the aqueous environment and the hydrophobic elements are sequestered, isolated from contact with polar components.

Thus, they reasoned that membrane proteins in a cell will assume globular (folded) conformations, due to hydrophobic and hydrophilic amino acid residues interacting with each other and the solvent environment, not the unfolded structures suggested by the DDR model. Similarly, membrane proteins will not be positioned to prevent contact between the polar head groups of membrane lipids and the aqueous cytoplasm.

So, if membrane proteins are globular and not layered on top of the membrane, where are they? How are they associated with the membrane?

The mosaic element of Singer and Nicolson's (1972) fluid mosaic model answered these questions. According to this model, membrane proteins come in two forms: **peripheral proteins**, which are dissolved in the cytoplasm and relatively loosely associated with the surface of the membrane, and **integral proteins**, which are integrated into the lipid matrix itself, to create a protein-phospholipid mosaic (Figure 2; Singer and Nicolson, 1972).



Original figure from Singer and Nicolson (1972) depicting membrane cross section with integral proteins in the phospholipid bilayer mosaic. Phospholipids are depicted as spheres with tails, proteins as embedded shaded, globular objects. Peripheral proteins, which would be situated at, not in, the membrane surface, are not shown. Recall that both surfaces of this membrane intercept an aqueous environment either the cytoplasm and/or the interstitial fluid. Transmembrane protein spanning entire membrane on left.

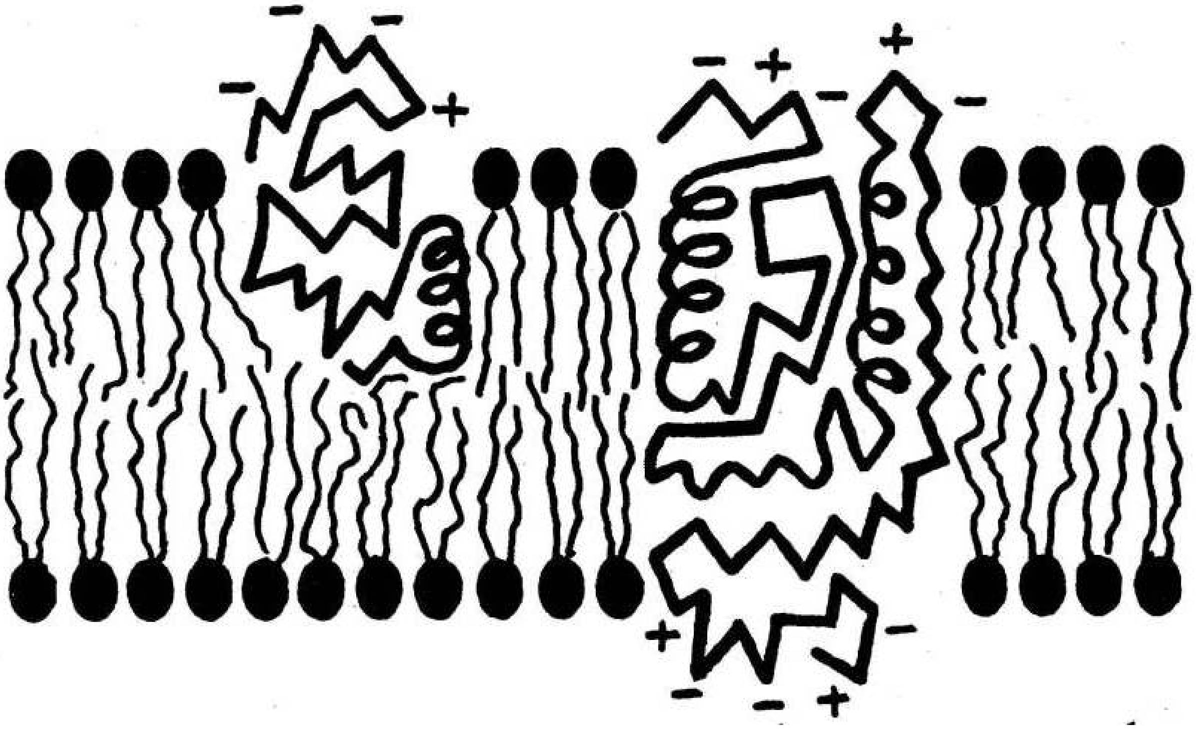
Singer and Nicolson (1972) supported these categories of proteins and their physical arrangement with both physical and biochemical evidence. For example, researchers had successfully separated the bilayers of frozen plasma membranes from a variety of sources including vacuoles, nuclei, chloroplasts, mitochondria and bacteria to reveal proteins embedded within (Singer and Nicolson, 1972). Similarly, evidence had also emerged to support the existence of transmembrane proteins, proteins that traversed the entire plasma membrane and extended into the aqueous environment on either side of the membrane (Figure 2).

Clear data supporting the predicted biochemical structure of integral proteins was harder to gain, however, and would only follow many years after the publication of the model. What was the biochemical structure of these proteins predicted to be?

Consider the energetic principles and molecular interactions on which Singer and Nicolson's model is based. Use your understanding of how these principles influence the structure and organization of individual polypeptides and the structural components of cells to answer the following questions.

1. Examine Figure 2. Predict biochemical properties (for example the hydrophobic or hydrophilic regions) of an integral protein versus those of a peripheral protein. Please be sure to explain your reasoning.

2. How do your predictions of the biochemical nature of integral proteins compare to Singer and Nicolson's predictions (Figure 3) adapted from a figure published by Lenard and Singer in 1966? If your predictions differ, please be sure to explain how and why they do.



Original figure from Singer and Nicolson (1972) depicting membrane cross section with integral proteins in the phospholipid bilayer. The ionic and polar portions of the proteins, as indicated by the +/- signs, contact the aqueous solutions (cytoplasm and/or interstitial fluid) surrounding the lipid bilayer. The membrane spanning or inserted region of the protein is non-polar/hydrophobic and therefore lacks charge as indicated by the absence of +/- symbols.

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The Fluid Mosaic Model - Determining the Matrix

Singer and Nicolson (1972) described the plasma membrane as a mosaic of proteins and phospholipids in a fluid phospholipid matrix. Proteins, however, form a substantial component of the membrane as Singer and Nicolson knew so what evidence suggested the matrix was lipid rather than protein in structure?

As is true of all good science, these two alternative models of the membrane matrix generated different testable predictions (hypotheses). Previous work had shown that the lipids of membranes behaved as a fluid under physiological conditions (Singer and Nicolson, 1972). Thus, if integral proteins were embedded, unanchored in a fluid matrix, then they would diffuse laterally through membrane surfaces much as dye particles diffuse through water. As a result, proteins would be randomly distributed on the surface of a membrane and, if monitored, would redistribute over time. That is, the distribution of proteins would be random and **dynamic** in time (Singer and Nicolson, 1972).

In contrast, if proteins formed the matrix in which lipids were dispersed, noncovalent bonds between proteins would inhibit diffusive protein movement and make the membrane relatively ridged. Consequently, proteins would likely be distributed in a regular, not random, pattern across the surface of the membrane and this distribution would be **static** (unchanging) not dynamic in time (Singer and Nicolson, 1972).

Prior to the 1972 *Science* publication, Singer's lab had developed a technique to test these predictions focusing, in particular, on the spatial distribution of proteins within the cell membrane at a single moment in time. Which model of the membrane matrix did their results support? Answer the questions below to find out!

1. Imagine you could see the proteins on the surface of a cell membrane sufficiently well to determine how they were distributed (spread). For each model above, sketch the predicted distribution of proteins on the surface of a cell membrane. That is, draw a cell membrane and add proteins to it in a pattern you might expect to see according to each hypothesis. Clearly label your diagrams.

Now let's turn your descriptive or **qualitative** predictions into measurement based or **quantitative** predictions.

2. Carefully compare your two drawings illustrating the expected distribution of proteins based on each model of the membrane matrix. What could you **measure** to enable you to determine numerically whether proteins are randomly or regularly spread across a cell's surface? Why? Please explain.

A reasonable variable to measure would be the distance between any two adjacent proteins. Take a look at your sketches again. On each roughly measure the distances between 50 pairs of adjacent proteins. Measure only uninterrupted distances; those for which the measurement path does not cross another protein. Record the distances in two parallel columns of numbers, one for each sketch corresponding to each model of membrane matrix structure.

3. Compare the two columns of measurements. Do they differ in anyway? If so, how? Please describe the nature of their differences.

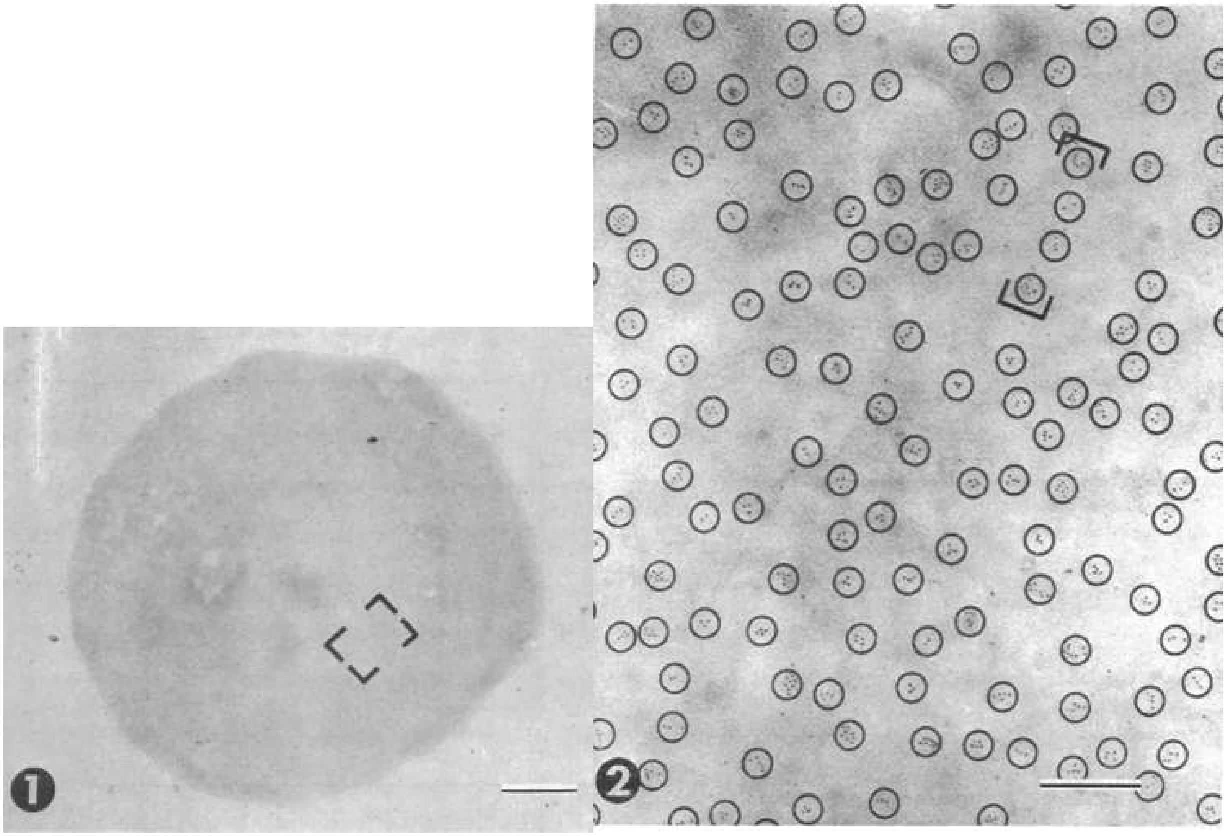
A good way to visually represent the differences between these two sets of measurements is to build a histogram, a.k.a. a frequency distribution. Recall that a frequency distribution, like the one found here, is a figure relating the number of times particular observations (values or discrete traits like eye color for example) appear in a sample.

For this investigation, building two hypothetical frequency distributions one per model, will give you expected distributions of measurements to which you can compare actual measurements of inter-protein distances in order to determine whether proteins appear to be randomly or regularly distributed on the surface of a cell membrane.

4. To build a hypothetical frequency distribution, return to your sketches and two hypothetical columns of numbers. Total the number of times you observe each particular distance. For example, note the number of times you measured a distance of 1 unit between two proteins, 2 units, 3 units etc. up to a maximum number of units you observed. Finally graph these totals by placing each unit of distance (ex. 1, 2, 3, etc.) on the x-axis and number

of times you expect to see each unit of distance on the y-axis. Produce one such histogram for each sketch.

Now you are prepared to compare the results you expect to see, i.e. your hypothetical frequency distributions, to the actual results from an experiment in which Nicolson, Masouredis and Singer (1971) stained the surface of human erythrocytes for the Rh protein (Figure 1).



Original figures from Nicolson *et al.* (1971) showing electron micrographs (photographs) of 1) a flattened plasma membrane retrieved from a lysed (ruptured) human erythrocyte and 2) bracketed region from figure 1.1 magnified to reveal stained, human Rh proteins on surface of the erythrocyte's plasma membrane. Each circle encloses a single Rh protein. Bar is 2um in length.

Examine Figure 1.2. Do these proteins look randomly or regularly distributed to you? To test your opinion as well as the two models of membrane matrix structure, answer the questions below.

To determine whether Rh proteins on the surface of human erythrocytes are randomly or regularly distributed, you need to build a histogram describing the frequency with which different distances among proteins occur on this plasma membrane.

5. To do this, measure the distances between 50 pairs of proteins by choosing one protein at random and measuring the distance from this protein to all neighboring proteins for which there is a direct line of sight (i.e. you do not have to measure across an intervening protein). Repeat this process until you have recorded 50 distances. Measure distance anyway you like as long as you are consistent and do not measure the same distance twice. Record your measurements in a spread sheet like Excel.

6. Organize your data into a histogram. To do this in Excel, first create the categories (bins) that will form the x-axis. In the column next to your data, create categories that reflect the range of observations you made in one unit increments. For example, if the smallest measured distance was 1 and the largest 31, create a column of numbers 1,2,3, 4...all the way to 31. These are your bins for tallying the number of measurements of each length. Second, go to the tools menu, select the data analysis option (if not present select add-ins and highlight the analysis tool pak box), and the histogram function. Select the input range box and highlight your column of measurements, then select the bin range box and highlight the bins you composed. Finally, select the output range box and select an empty cell where you would your tallied data to appear. Highlight the chart output box at the bottom of the menu and click ok. You should see a figure titled *'histogram'* and two associated columns of numbers one labeled bin, the other frequency. Click on the bars in the chart and the data columns describing the values on the x (bin) and y (frequency) axes respectively should become highlighted.

7. Write a figure legend for your histogram that describes the data illustrated in your figure. Your legend should simply relate what is shown but not interpret the data in anyway.

8. Compare your histogram to the histograms you produced for question 4. Do you think Rh proteins are randomly or regularly distributed on the

surface of erythrocytes? Why or why not? Please support your explanation and conclusion with evidence.

9. Imagine you are Nicolson, Masouredis and Singer. Which model of matrix structure, lipid or protein, do you interpret your data to support? Why? Please explain.

10. Are you comfortable with the conclusion you drew in question 9 on the basis of this data? Why or why not? Please explain. If you are not, please explain one way in which you could increase your confidence in your conclusion.

Works Cited

- Nicolson, G.L., S.P. Masouredis, and S.J. Singer. 1971. Quantitative two dimensional ultrastructural distribution of Rho(D) antigenic sites on human erythrocyte membranes. *Proceedings of the National Academy of Sciences*. 68:1416-1420.
- Singer, S.J. and G.L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science*. 175:720-731.

The Fluic Mosaic Model - The Fluid Lipid Matrix

Singer and Nicolson (1972) described the plasma membrane as a mosaic of proteins and phospholipids in a fluid phospholipid matrix. To do this, however, they needed to a) provide evidence that membrane proteins were actually embedded within the membrane and b) determine whether the membrane's matrix was protein or lipid in structure as both types of biological molecules constitute major components of the membrane.

Experimental results published in 1971 suggested that proteins were randomly, not regularly, distributed as predicted by the lipid model (Nicolson, Masouredis, and Singer, 1971; Nicolson, Hyman, and Singer, 1971). However, these results did not bear on the second prediction of the lipid matrix model: ,that the distribution of integral proteins would be dynamic, that is, would change through time as the proteins diffused through the fluid lipid matrix (Singer and Nicolson, 1972).

To support this second prediction, Singer and Nicolson (1972) cited an elegant experiment published in 1970 by L.D. Frye and M. Edidin. The goal of Frye and Edidin's study had been to demonstrate that the plasma membrane was fluid in nature, an attribute they felt had been ignored by certain models of membrane structure. As they wrote in the introduction to their paper,

> The surface of membranes of animal cells rapidly change shape as the cells move, form pseduopods, or ingest materials from their environment. These rapid changes in shape suggest that the plasma membrane itself is fluid, rather than rigid in character, and that at least some of its component macromolecules are free to move relative to one another within the fluid. We have attempted to demonstrate such freedom of movement using specific antigen markers of 2 unlike unlike cell surfaces. (Frye and Edidin, 1970, p.320)

To demonstrate the membrane's fluidity, Frye and Edidin created hybrid cells or **‘heterokaryons’** by fusing a human cell with a mouse cell to form a single cell with a continuous plasma membrane containing both human and mouse specific integral proteins. After fusion, the distribution of proteins on the cell surface was tracked for several hours using chemical stains that differentiated human from mouse proteins. The spatial distribution of proteins in these ‘double-stained cells’ were compared to control preparations of human-human fused and mouse-mouse fused cells which stained exclusively the color specific to human or mouse proteins respectively (Frye and Edidin, 1970). Frye and Edidin’s results appear in Figure 1 below.

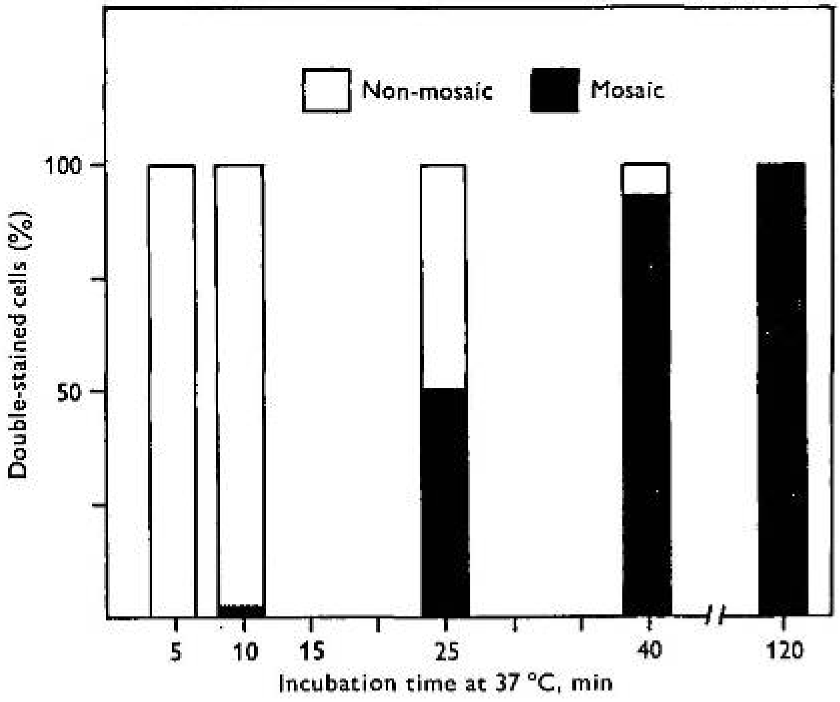


Fig. 1. Appearance of completely double-staining (mosaic) cells in the population of fused cells.

Original figure from Frye and Edinin (1970) showing the relationship between the percentage of double-stained (i.e. hybrid) cells exhibiting a mosaic (black) or non-mosaic (white) distribution of human and mouse proteins on their surface versus incubation time (in minutes at 37o C). Mosaic cells are those in which human and mouse proteins are completely intermixed across the surface of the cell. In the non-mosaic condition, mouse and human proteins are not mixed but rather spatially segregated with human proteins concentrated in one half and mouse in the other half of the cell. This is the distribution of proteins at the time of cell fusion.

1. Carefully examine the figure legend, axes labels and data. Summarize the results of this experiment as **depicted in the figure** above in a sentence or two. Be sure to describe only what the figure shows.

2. What do their results suggest about the position of proteins within the cell membrane over time? Why? Please explain.

3. Review the experimental control described in the paragraph above. Please explain why the control is important to the interpretation of the results in Figure 1.

4. Singer and Nicolson (1972) cite this study as evidence that the membrane behaves as a fluid through which proteins can diffuse. Review the results above, do you think they can be unequivocally interpreted as evidence for a fluid cell membrane or could these same results be caused by alternative cellular mechanisms? Please explain. If you conclude that alternative explanations are possible, please describe them.

5. Choose one of the models (explanations) you described in question 4 and briefly outline an experiment to test it. Be sure to explain what variable(s) you will measure and what results will support or refute the model.

Ultimately, Frye and Edidin (1970) were able to convincingly support the model that mosaic formation was due to the diffusion of stained proteins and thus, by implication, that the membrane behaved as a fluid as opposed to a rigid structure. To do so, they had to systematically eliminate several other models offering alternative mechanisms for the observed redistribution of proteins. These included the possibilities that

1. new proteins were rapidly synthesized and inserted into the membrane over the course of the experiment.
2. proteins were being removed from the surface in one location and reinserted in another.
3. proteins synthesized in both the mouse and human cell prior to cell fusion were being inserted into the membrane after fusion.

To eliminate these alternative models, Frye and Edidin (1970) conducted additional experiments in which they treated the cells with chemicals that inhibited protein or ATP synthesis either before fusion (to test model 3) or after fusion (to test models 1 and 2). None of these treatments impeded protein redistribution and thus, mosaic formation, as expected if the alternative models were correct.

Temperature at which the cells were incubated did, however, affect rates of mosaic formation (Frye and Edidin, 1970). Was this outcome consistent with the explanation that protein redistribution results from diffusion through a fluid membrane? Answer the questions below to find out.

1. Consider the process of diffusion. How would you expect temperature to affect the rate at which molecules diffuse? Why? Please explain with reference to the process by which diffusion works.

2. Given the expectation articulated in question 1, predict how you would expect temperature to affect the number of mosaic cells formed after 40 minutes of incubation if protein movement was diffusion driven. To do this, imagine incubating a set of newly formed heterokaryons at a constant, preselected temperature and counting the number of mosaics after 40 minutes and then repeating this procedure for a series of predetermined temperatures between 0 and 37 degrees Celsius.

3. Convert your prediction from question 2 into a figure (graph) with temperature on the x-axis and % of cells exhibiting mosaicism after 40 minutes of incubation on the y-axis.

4. Consider the expected relationship between temperature and mosaic formation described in your answer to question 2. What types of results

(relationships between temperature and mosaic formation) would not support this model? Please explain.

5. Sketch the null models described in question 4 on the figure produced for question 2.

Frye and Edidin's (1970) results describing the relationship between temperature of incubation and mosaic formation appear in Figure 2.

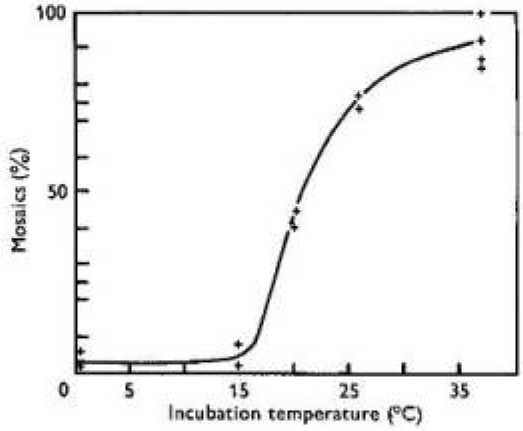


Fig. 2. Effect of temperature on the appearance of mosaic cells within 40 min of cell fusion.

Original figure from Frye and Edidin (1970) describing the relationship between the percentage cells exhibiting mosaicism after a 40 minute incubation at temperatures (degrees Celsius) indicated by + symbols. Curve fit to data.

1. Carefully examine the figure legend, axes labels and data. Summarize the results of this experiment as depicted in the figure above in a sentence or two. Be sure to describe only what the figure shows.

2. Compare the data to the figures produced for questions 3 and 5 above. Which of your predictions, if any, do these results appear to support? Why?

Please explain.

3. Do these results support a fluid model of the cell membrane in which proteins move by diffusion? Yes or no? Please explain.

Works Cited

- Frye, L.D. and M. Edidin. 1970. The rapid intermixing of cell surface antigens after formation of mouse-human heterokaryons. *Journal of Cell Science.* 7:319-335.
- Nicolson, G.L., R. Hyman, and S.J. Singer. 1971. The two-dimensional topographic distribution of H-2 histocompatibility alloantigens on mouse red blood cell membranes. *The Journal of Cell Biology*. 50:905-910.
- Nicolson, G.L., S.P. Masouredis, and S.J. Singer. 1971. Quantitative two-dimensional ultrastructural distribution of Rho(D) antigenic sites on human erthryocyte membranes. *Proceedings of the National Academy of Sciences.* 68:1416-1420.
- Singer, S.J. and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science*. 175: 720-731.

Summarize and Test Your Understanding of the Fluid Mosaic Model of Membrane Structure

Singer and Nicholson's (1972) fluid mosaic model, which has been only very slightly modified despite an additional 35 years of discovery, is the now the accepted model for plasma membrane structure.

1. On a piece of paper, describe your understanding of the biochemical structure and organization of the plasma membrane. A superior response will include the molecular interactions responsible for its physical organization.

2. Recall that the biochemical composition and structural organization of the plasma membrane render it 'semi-permeable' as Overton observed over 100 years ago. That is, some molecules can diffuse through it while others cannot. Review the list of molecules below and for each one propose and explain a reason that references the structure of the membrane for the indicated permeability property.

Exercise:

Problem: Hydrogen ion (H+): Impermeable.

Solution:

Ions are charged and are hydrophobic. They cannot dissolve in the hydrophobic interior of the plasma membrane and so cannot diffuse directly through the membrane.

Exercise:

Problem: Oxygen (O2): Permeable.

Solution:

Oxygen is a non-polar molecule because electrons are shared equally by both atoms. Therefore, it can diffuse directly through the hydrophobic interior of the plasma membrane.

Exercise:

Problem: Carbon dioxide (CO2): Permeable.

Solution:

Although the chemical bonds of this molecular are polar because the oxygen atoms pull electrons away from the carbon, the oxygen atoms are arranged on either side of the carbon in such a way that it distributes the charge more or less evenly across the surface of the molecule. Thus, the molecule as a whole is non-polar. As such, the molecule is able to diffuse directly through the hydrophobic interior of the plasma membrane.

Exercise:

Problem: Glucose: Impermeable.

Solution:

The hydroxyl groups on the molecule are charged and therefore non-polar. These charges prevent glucose from diffusing directly through the hydrophobic interior of the plasma membrane.

Exercise:

Problem: Calcium ion (Ca2+): Impermeable.

Solution:

The calcium ion, like the hydrogen ion, is charged, Consequently it cannot diffuse directly through the hydrophobic interior of the membrane.

Exercise:

Problem: Water (H2O): Permeable.

Solution:

Oddly water can diffuse directly through the membrane even though it is a highly polar molecule due to the unequal charge distribution that arises from the oxygen atom pulling electrons away from the two hydrogen atoms. Its very small size is believed to be important to its ability to diffuse directly through the membrane.

Works Cited

- Singer, S.J. and G. L. Nicolson. 1972. The fluid mosaic model of the structure of cell membranes. *Science*. 175: 720-731.